

*Application No. 10/814,244*

*Reply to Office Action dated January 2, 2008*

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**Amendment of the claims:**

**This listing of claims will replace all prior versions, and listings, of claims in the application.**

**LISTING OF CLAIMS**

1. **(currently amended)** A method for mass spectrometric analysis of a sample comprising at least two antiretroviral drugs from at least two classes of antiretroviral drugs, the method comprising the steps:
  - (a) providing a sample comprising at least two antiretroviral drugs from at least two classes of antiretroviral drugs;
  - (b) introducing the sample comprising at least two antiretroviral drugs from at least two classes of antiretroviral drugs into a mass spectrometer; and
  - (c) analyzing the sample using the mass spectrometer,wherein the method does not include any evaporation and reconstitution steps.
2. **(original)** The method according to claim 1 wherein the mass spectrometer is a tandem-mass spectrometer.
3. **(original)** The method of claim 1 further comprising a step of deproteinating the sample.
4. **(original)** The method according to claim 1 wherein the classes of antiretroviral drugs are selected from the group consisting of PIs, NRTIs, NtRTIs, NNRTIs and FIs.
5. **(original)** The method according to claim 1 wherein the antiretroviral drugs are selected from the group consisting of amprenavir, indinavir, nelfinavir, ritonavir, saquinavir, lopinavir, abacavir, didanosine, lamivudine, stavudine, zalcitabine, zidovudine, delavirdine, efavirenz, nevirapine, tanofavir, atazanavir, peptide T and T-20.
6. **(original)** The method according to claim 1 wherein the sample containing at least two antiretroviral drugs is obtained from a biological matrix selected from the group consisting of plasma, serum, urine and saliva.
7. **(original)** The method of claim 6 wherein the biological matrix is plasma.
8. **(original)** The method of claim 6 wherein the biological matrix is serum.
9. **(original)** The method of claim 6 wherein the biological matrix is saliva.

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10. **(original)** The method according to claim 1 wherein size of the sample containing at least two antiretroviral drugs is about 80  $\mu$ L.
11. **(original)** The method according to claim 3 wherein the step of deproteinating the sample comprises:
  - (a) adding acetonitrile to the sample;
  - (b) vortexing the sample; and
  - (c) subjecting the sample to centrifugation.
12. **(original)** The method according to claim 3 wherein the step of deproteinating the sample comprises subjecting the sample to precipitation with an agent selected from the group consisting of methanol, ethanol and salt.
13. **(original)** The method of claim 1 further comprising a step of cleaning the sample.
14. **(original)** The method according to claim 13 wherein the step of cleaning the sample comprises introducing the sample to a chromatography apparatus and eluting the sample.
15. **(currently amended)** The method according to claim 2 wherein the tandem-mass spectrometer is selected from the group consisting of API 2000™, API 3000™ and API 4000™.
16. **(original)** The method according to claim 1 wherein the step of analyzing the sample using a mass spectrometer comprises a step of atmospheric pressure chemical ionization using a heated nebulizer.
17. **(original)** The method according to claim 1 wherein the step of analyzing the sample using a mass spectrometer comprises multiple reaction monitoring.
18. **(original)** The method according to claim 1 which does not include chromatographic separation of the antiretroviral drugs.
19. **(original)** The method according to claim 1 wherein the sample comprises a plurality of antiretroviral drugs and they are analyzed simultaneously.
20. **(original)** The method according to claim 1 wherein the sample comprises a plurality of antiretroviral drugs and they are analyzed sequentially.
21. **(currently amended)** A method for therapeutic drug monitoring in patients with HIV infection, comprising:

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- (a) providing a sample comprising at least two antiretroviral drugs from at least two classes of antiretroviral drugs;
  - (b) introducing the sample comprising at least two antiretroviral drugs from at least two classes of antiretroviral drugs into a mass spectrometer; and
  - (c) analyzing the sample using the mass spectrometer,  
wherein the method does not include any evaporation and reconstitution steps.
22. **(original)** The method according to claim 21 wherein the mass spectrometer is a tandem-mass spectrometer.
23. **(original)** The method of claim 21 further comprising a step of deproteinating the sample.
24. **(original)** The method according to claim 21 wherein the classes of antiretroviral drug is selected from the group consisting of PIs, NRTIs, NtRTIs, NNRTIs and FIs.
25. **(original)** The method according to claim 21 wherein the antiretroviral drug is selected from the group consisting of amprenavir, indinavir, nelfinavir, ritonavir, saquinavir, lopinavir, abacavir, didanosine, lamivudine, stavudine, zalcitabine, zidovudine, delavirdine, efavirenz, nevirapine, tanofavir, atazanavir, peptide T and T-20.
26. **(original)** The method according to claim 21 wherein the sample containing at least two antiretroviral drugs is obtained from a biological matrix selected from the group consisting of plasma, serum, urine and saliva.
27. **(original)** The method of claim 21 further comprising a step of cleaning the sample.
28. **(original)** A system for the mass spectrometric analysis of a sample comprising at least two antiretroviral drugs from at least two classes of antiretroviral drugs, comprising:
  - (a) reagents for deproteinating the sample;
  - (b) reagents for analyzing the sample by mass spectrometry; and
  - (c) a mass spectrometer.
29. **(original)** A kit for use in mass spectrometric analysis of a sample comprising at least two antiretroviral drugs from at least two classes of antiretroviral drugs, comprising:
  - (a) reagents for deproteinating the sample;
  - (b) reagents for analyzing the sample by mass spectrometry;
  - (c) instructions for analyzing the sample using a mass spectrometer.
30. **(original)** The kit according to claim 29 further comprising:
  - (a) mobile phase solutions;

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- (b) a chromatography column; and
- (c) a quality control specimen.

31. **(currently amended)** Use of a mass spectrometer for sequentially or simultaneously analyzing a sample containing at least two antiretroviral drugs from at least two classes of antiretroviral drugs, comprising the steps:

- (a) providing a sample comprising at least two antiretroviral drugs from at least two classes of antiretroviral drugs;
  - (b) introducing the sample comprising at least two antiretroviral drugs from at least two classes of antiretroviral drugs into a mass spectrometer; and
  - (c) analyzing the sample using the mass spectrometer,
- wherein the use does not include any evaporation and reconstitution steps.

32. **(original)** The use of claim 31 wherein the mass spectrometer is a tandem mass spectrometer.

33. **(new)** A method for mass spectrometric analysis of free antiretroviral drug concentration in a sample comprising or suspected of comprising an antiretroviral drug, the method comprising the steps:

- (a) providing the sample;
- (b) purifying the sample;
- (c) introducing the sample into a mass spectrometer; and
- (d) analyzing the sample using the mass spectrometer.

34. **(new)** The method according to claim 1 wherein size of the sample introduced into the mass spectrometer is about 50  $\mu$ L.

35. **(new)** A method for therapeutic drug monitoring of free antiretroviral drug concentration in a patient, comprising:

- (a) providing a sample from the patient comprising or suspected of comprising an antiretroviral drug;
- (b) purifying the sample;
- (c) introducing the sample into a mass spectrometer; and
- (d) analyzing the sample using the mass spectrometer.

36. **(new)** The method of claims 33 or 35 wherein the step of purifying the sample comprises equilibrium dialysis or use of molecular cutoff filters.

37. **(new)** The method of claim 36 wherein the molecular cutoff filter is selected from the group consisting of Amicon Centrifree micropartition system and Worthington Diagnostics "ultrafree" system.

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38. (new) A method for mass spectrometric analysis of a sample comprising or suspected of comprising tenofovir, the method comprising the steps:

(a) providing a sample comprising tenofovir or suspected of comprising tenofovir;

(b) introducing the sample comprising tenofovir or suspected of comprising tenofovir into a mass spectrometer; and

(c) analyzing the sample using the mass spectrometer.

39. (new) The method of claim 1 wherein the antiretroviral drugs include drugs from each of PIs, NTRIs, NtRTIs and NNTRIs.